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Molecular phylogenetic relationships of *Inchoatia* taxa

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The molecular phylogenetic relationships between nominal (sub)species within the Greek land snail genus *Inchoatia* are examined.

Introduction

The genus *Inchoatia* was introduced by Gittenberger & Uit de Weerd (2006a, b) on the basis of molecular phylogenetic analyses (Uit de Weerd et al., 2004). In its present form the genus consists of four nominal species: *I. haussknechti* (O. Boettger, 1886), *I. inchoata* (O. Boettger, 1889), *I. megdova* (H. Nordsieck, 1974) and *I. regina* (H. Nordsieck, 1972). Besides synapomorphic nucleotide substitutions, these four taxa share a similar shell shape and have more or less continuous ranges. According to the molecular data, the major division within *Inchoatia* is between (1) an *I. inchoata* + *I. regina* subclade, and (2) an *I. haussknechti* + *I. megdova* subclade. This pattern is congruent (1) with genital anatomical differences between the two subclades, previously used to place them in separate genera, viz. *Sericata* and *Carinigera* (see Nordsieck, 1974), and (2) with conchological similarities between the species within each subclade.

A major problem has been the morphological and geographical delimitation of the species within each subclade. Nordsieck (1972) recognizes *I. regina* as a separate species with a range situated amidst those of the *I. inchoata* subspecies, stating (p.16) that the differences with *I. inchoata* are sufficiently large ("groß genug"). Similarly, *I. haussknechti* and *I. megdova* have a mosaic distribution. However, where their populations come geographically close, without contact zones as far as known, morphological convergence is not seen. For more details, see Gittenberger & Uit de Weerd (2009).

The molecular phylogenetic study by Uit de Weerd et al. (2004) was not designed to answer questions about the status of the speciesgroup taxa within *Inchoatia*. Only four samples, representing each of the four nominal species, were included: *I. haussknechti alticola* (H. Nordsieck, 1974), *I. inchoata inchoata* (O. Boettger, 1889), *I. megdova tavropodensis* (Fauer, 1993), and *I. regina* (H. Nordsieck, 1972). Moreover, according to Nordsieck (2007: 109), "[*I.*] *haussknechti alticola* H. Nordsieck and [*I.*] *megdova tavropodensis* Fauer, belong to one and the same species". Referring to an unpublished revision, Nordsieck (2007) recognizes not two, but four species within the *I. haussknechti* - *I. megdova*

assemblage (see Table 1). Thus the diversity within this assemblage may have been insufficiently sampled by Uit de Weerd et al. (2004).

As a step towards a more objectively founded taxonomic and biogeographical delimitation of taxa within *Inchoatia*, we determined cytochrome c oxidase subunit 1 (COI) nucleotide sequences of multiple samples of seven additional subspecies within the *Inchoatia* clade, and conducted phylogenetic analyses.

Materials and methods

In order to unravel the interrelationships among the taxa within the genus, we supplemented the four *Inchoatia* sequences of Uit de Weerd et al. (2004) with 19 additional partial cytochrome c oxidase subunit 1 (COI) sequences (see Table 2), following the same procedures. With this increased sampling all three subspecies of *I. inchoata* are represented, as are three subspecies of *I. haussknechti* and three subspecies of *I. megdova* (see Gittenberger & Uit de Weerd, 2009). As an outgroup four previously determined partial COI sequences (AY425562-AY425565) of the closely related (Uit de Weerd et al., 2004) genus *Cristataria* were used. All phylogenetic analyses were performed using

Table 1. Taxa recognized within the *I. haussknechti - I. megdova* assemblage according to Nordsieck (1974) and Fauer (1993) (left), and modifications suggested by Nordsieck (2007) (right).

2 species (Nordsieck, 1974; Hausdorf, 1987; Fauer, 1993)		**4 species (Nordsieck, 2007)**
species	**subspecies**	**placed in**
[I.] haussknechti	*[I.] h. alticola* (H. Nordsieck, 1974)	*[I.] semilaevis*
	[I.] h. haussknechti (O. Boettger 1886)	*[I.] haussknechti*
	[I.] h. hiltrudae (H. Nordsieck, 1974)	*[I.] hiltrudae*
	[I.] h. refuga (Westerlund 1894)	*[I.] haussknechti*
	[I.] h. semilaevis (O. Boettger 1889)	*[I.] semilaevis*
[I.] megdova	*[I.] m. megdova* (H. Nordsieck, 1974)	*[I.] megdova*
	[I.] m. palatalifera (Hausdorf, 1987)	*[I.] megdova*
	[I.] m. tavropodensis (Fauer 1993)	*[I.] semilaevis*

Table 2. Information on the populations sampled as an ingroup in the phylogenetic analyses of the genus *Inchoatia*. (*) COI sequences obtained by Uit de Weerd et al., 2004. For information regarding the outgroup samples, see Uit de Weerd et al., 2004.

No.	**(sub)species**	**GenBank Acc. No.**	**UTM**	**co-ordinates**
1	*Inchoatia haussknechti alticola*	AY425555*	EJ6910	38º57'N 21º48'E
2	*Inchoatia haussknechti alticola*	DQ105075	EJ7111	38º57'N 21º49'E
3	*Inchoatia haussknechti haussknechti*	DQ105089; DQ105090	EJ9225	39º04'N 22º04'E
4	*Inchoatia haussknechti hiltrudae*	DQ105076; DQ105077; DQ105078	EJ5168	39º28'N 21º36'E
5	*Inchoatia inchoata inchoata*	AY425586*; DQ105079	DJ7233	39º09'N 20º41'E
6	*Inchoatia inchoata klemmi*	DQ105080; DQ105081	EJ0062	39º25'N 21º00'E
7	*Inchoatia inchoata paramythica*	DQ105082; DQ105083	DJ6753	39º20'N 20º37'E
8	*Inchoatia megdova bruggeni*	DQ105091; DQ105092; DQ105093	EJ4669	39º29'N 21º32'E
9	*Inchoatia megdova megdova*	DQ105084; DQ105085; DQ105086	EJ5209	38º56'N 21º36'E
10	*Inchoatia megdova tavropodensis*	AY425556*; DQ105087	EJ5910	38º57'N 21º41'E
11	*Inchoatia inchoata regina*	AY425589*; DQ105088	DJ8647	39º17'N 20º51'E

PAUP* 4.0b10 (Swofford, 2002). Prior to the analyses, we made certain that the base frequencies for each of the three codon positions were non-inhomogeneous across taxa (Chi-square test). Heuristic MP searches consisted of 1000 random addition replicates (tree bisection and reconnection, steepest descent). In addition, an MP bootstrap analysis consisting of 10,000 replicates was performed. Each replicate consisted of 10 random addition replicates (tree bisection and reconnection, steepest descent).

Results and discussion

The phylogenetic analyses retrieved two MP trees (score 562), the strict consensus of which is shown in figure 1. This tree confirms that *Inchoatia* consists of two main subclades, i.e. subclade (1) consisting of *Inchoatia inchoata* en *Inchoatia regina*, and subclade (2) with *Inchoatia haussknechti* and *Inchoatia megdova*.

Within subclade 1, *Inchoatia regina* is nested in *I. inchoata*. As a sistergroup to *Inchoatia inchoata klemmi* (H. Nordsieck, 1972), *I. regina* constitutes two successive highly (≥ 99% bootstap) supported clades with *I. inchoata* subspecies. This result is congruent with the conchological similarities between *I. regina* and *I. inchoata klemmi*, which are

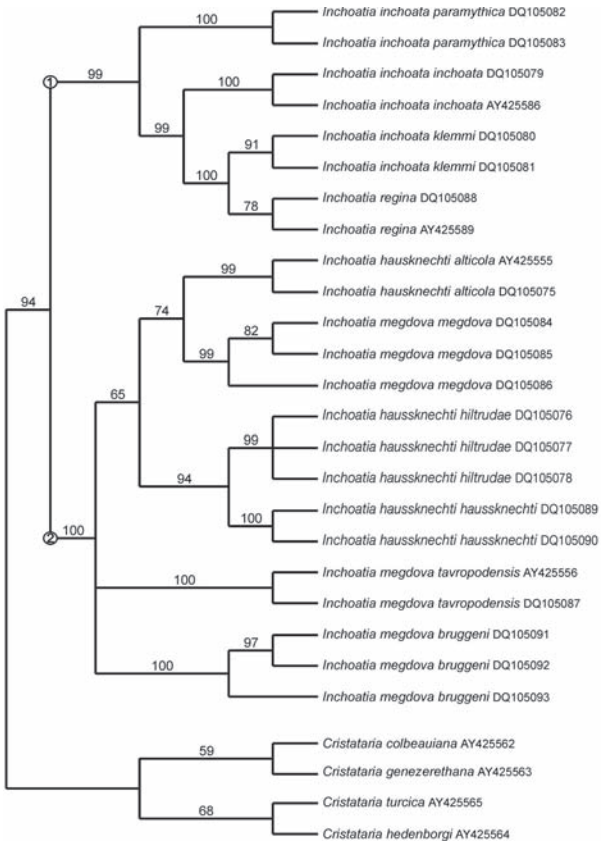

Fig. 1. Strict consensus of two MP trees for the COI dataset. Numbers along branches indicate bootstrap values. Encircles numbers refer to subclade 1 and subclade 2. GenBank accession numbers are given behind taxon names.

also biogeographical neighbours. We therefore see no reason to attach a special status to *I. regina* as a separate species next to *I. inchoata*, and propose to classify this taxon as *Inchoatia inchoata regina*.

In subclade 2, the phylogenetic status of the species *Inchoatia megdova* and *I. haussknechti* is far less clear. Although the COI sequences of the *I. megdova megdova* population are nested among the *I. haussknechti* sequences, this nested position is comparatively weakly supported (bootstrap value 74% and 65%). The remaining two *I. megdova* subspecies sampled are placed on separate branches within a basal trichotomy in subclade 2.

The three most parsimonious trees (score 576) congruent with the classification suggested by Nordsieck (2007) are significantly (one-sided Templeton test: $P<0.01$) less supported by the COI data than either MP tree. This is not consistently the case (one-sided Templeton test: $P = 0.11$; $P = 0.04$) for the most parsimonious tree (score 568) congruent with the prevalent classification, in which *I. megdova* and *I. haussknechti* are both monophyletic and together accommodate all subspecies within the assemblage, including *I. megdova bruggeni* Gittenberger & Uit de Weerd, 2009. In the absence of evidence to the contrary, we therefore propose to maintain that latter classification.

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